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GROWTH HORMONE ASSAYS: CLINICAL RESULTS OBTAINED WITH COMMERCIAL KITS.

Much attention is being directed to the incidence of growth hormone deficiency and limits of HGH-concentration in respons to stimuli are under debate. In this study 23 serum samples from four patients were obtained during various stimulation test and analyzed by means of the following kits: Pharmacia (RIA 100), CIS (SB-HGH-RIA equal to Sorin HGHK-2), Serono (HGH, RIA) and Hybritech (Tandem-R-HGH). All results were obtained in mU/l, the first three kits being standardized against WHO 66/217 and the last against HS 2243 E NIH. Pharmacia RIA-100 was the "in house method" (x) and the following correlations between the results obtained by other methods (y) were obtained:

CIS : $y = 1{,}99\ x - 4{,}25$ $r = 0{,}930$
Serono : $y = 1{,}04\ x + 0{,}59$ $r = 0{,}983$
Hybritech: $y = 0{,}809x - 1{,}17$ $r = 0{,}993$

Samples of biosynthetic HGH: B-HGH was diluted to 22.1 mU/l and Pituitary standard P-HGH diluted to 18.8 mU/l, both from Nordisk Gentofte gave following results (mU/l):

	B-HGH 1986(4IU/1,36mg)	P-HGH 1985(0,95IU/0,39mg)
Pharmacia	22,4	20,7
CIS	20,4	15,2
Serono	24,0	17,4
Hybritech	18,0	15,7

In conclusion methods based on one antibody-RIA (CIS=Sorin, Serono) gave the highest levels of HGH in plasma samples. Of the two IRMA-kits, Hybritech with two monoclonal antibodies gave the lowest results. Accordingly the dividing line between normal and partial deficiency is closely linked to the HGH assay chosen.

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DOES URINARY GROWTH HORMONE (GH) REFLECT PLASMA GROWTH HORMONE LEVELS?

In 40 children, a 24 h-plasma GH profile has been compared to urinary GH excretion. Plasma was assayed with a conventional radioimmunoassay (kit CEA). For urine, a radiometric assay has been developed with a solid-phase goat antibody for immunextraction and a I^{125} monoclonal antibody for quantification. The assay is insensitive to pH 5-8, NaCl/urea 0.1-0.5 mol and to sample volumes up to 10 ml. The sensitivity of 2 pg in the standard results in a detection limit of 1 pg/ml, if 2.5 ml of urine are used. Coefficients of variation are 10-14% for inter-, and 2.5-3.5% for intra-assay. Plasma GH was expressed as integrated concentration (IC, ng/ml/h) per 24 h or night/day periods, urinary GH as pg per 24 h or night/day and was related to creatinine. 1) Correlations for plasma were: IC 24 h to a) IC night 0.93, b) IC day 0.77. 2) Correlations for urine ($\pm$ creatinine): 24 h to a) day 0.73, b) night 0.8, 24 h/cr. to a) day/cr. 0.53, b) night/cr. 0.83. 3) Correlations for plasma to urine: Plasma IC 24 h to a) urine 24 h 0.45, b) urine 24 h/cr. 0.60, c) urine night/cr. 0.69. It can thus be concluded that urinary GH reflects plasma levels and a daily GH-production. GH-assays in urine are an additional help for the diagnosis of states of deficiency or excess and for the control of suppressive or stimulating therapies.

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URINARY GROWTH HORMONE (GH) - CLINICAL APPLICATION.

A radiometric assay for GH was applied to unprocessed urine (technical details previously described). A significant correlation between plasma profiles and urinary GH excretion has been found (i.e. 24 h plasma integrated concentrations (IC) to urine pg/24 h, r = 0.45. Plasma IC 24 h to night urine GH/creatinine 0.69, night plasma IC to night urine 0.58, for all correlations N = 40). In 155 24 h-urines of "normal" children (partly referred for suspected growth problems), a mean GH-excretion of 6.2 $\pm$ 6.5 ng/24 h (median 4.1, P. 10 1.3, P. 90 13.6) has been found. No clear relation was found to chronological age, bone age or puberty ratings. 10 patients with "precocious puberty" (5 idiopathic, 5 treated CAH) had a mean excretion of 9.7 $\pm$ 5.4 (median 8.4, P. 10 4.6, P. 90 19). In active acromegaly, the values varied from 73 to 500 ng/24 h. Patients with "complete" (N = 9) and "partial" (N = 7) GH-deficiency had a mean (median) excretion of 0.79 (0.75) and 3.3 (1.8) ng/24 h off therapy. The values increased to 6.2 (3.8) and 11.3 (10.5) ng/24 h during therapy (2 IU daily s.c.). Before more conclusions can be drawn, the important intraindividual variation of GH-excretion has to be considered. Nevertheless, urinary GH mirrors an actual GH-production over a set time and can be applied to clinical states with suspected "abnormal" GH-production or for therapy survey.

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MEASUREMENT OF URINARY GROWTH HORMONE (uGH) BY A SENSITIVE ENZYME IMMUNOMETRIC ASSAY (EIA) IN CHILDREN

An EIA developed for the measurement of serum GH, was adapted for the measurement of uGH. Due to the very low levels of GH, urine samples are routinely dialyzed and concentrated at least 15 fold by centrifugal ultrafiltration. Urine samples collected in plastic tubes containing BSA (1g/l) are stored at -20°C until analyzed for GH and creatinine (CR). Under these conditions GH is stable for at least 2 months. Mean recovery of GH after dialysis and concentration is 91.4%. The sensitivity of the assay is 0.4 pg/tube. The intraassay and interassay coefficients of variation are respectively 4.9% and 5.5% at a concentration of 4.5 pg/ml. The centrifugal ultrafiltration step requires approximately 45' per batch. Using this method we analyzed first-morning urine samples of healthy children and of children referred for short stature. The results in the healthy children vary from 5 to 100 ng GH/g CR. In children with documented GH deficiency results are <3 ng GH/g CR. In children with intermediate GH response to ITT and/or ARG results range from 3 to 6 ng GH/g CR. Furthermore, we found a highly significant correlation between uGH and peak serum GH during ITT or ARG provocation tests. From our results we conclude that the measurement of uGH in morning urine reflects night-GH production. The effectiveness of this parameter for diagnostic purpose has to be further studied, due to the large day to day variation of measured uGH in individual children.

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A SENSITIVE ELISA FOR THE MEASUREMENT OF GH IN SERUM, BLOOD AND URINE

A sensitive sandwich ELISA for GH was developed using guinea-pig (GP) polyclonal IgG to coat microtitre plates (which can be stored for up to 3 months). The standard was 22K biosynthetic hGH (2.97U/mg). Assay volume was 100µl which could include up to 20µl of serum, plasma or blood in buffer, (0.04mol/l sodium phosphate, pH7.4, containing human serum albumin 6g/l), or 100µl of dialysed urine. Total assay time was 16hrs. The conjugate was a peroxidase labelled Fab'-fragment of GP anti-hGH with O-phenylenediamine as the substrate for the enzymatic reaction. Optical density was read at 490nm. The detection limit was 0.5 nanounits/well. The working assay range using 20µl of serum was 0.05-15mU/l. The inter assay coefficients of variation were 6.8% at 0.15mU/l, 2.4% at 2.2mU/l and 6.9% at 11.6mU/l. The assay was compared to an IRMA (Pharmacia); the regression equation was Y (ELISA) = 0.967X (IRMA) - 0.84 with r = 0.996 for 18 samples ranging from 1 to 60mU/l.

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CONTINUOUS S.C. INFUSION OF GHRH (1-29) AUGMENTS GH SECRETION AND PROMOTES GROWTH OVER SIX MONTHS

We have previously shown in 14 normal adult males that an 8 day continuous s.c. infusion of GHRH (1-29) in a dose regimen of 60ng/kg/min efficiently augmented GH secretion (ESPE 1987). There was no evidence of desensitization of the hypothalamo-pituitary axis over a range of doses from 7.5-120ng/kg/min.

We now report a study of treatment of 8 children with continuous GHRH infusion for six months. All were short, slowly growing prepubertal children with peak GH responses to insulin-induced hypoglycaemia of 10-20mu/l. 24 hour GH profiles were performed before treatment, on Day 1, at 5 weeks, three months and six months into treatment. GHRH (1-29) was infused continuously subcutaneously at a rate of 60ng/kg/min. Sum of GH pulse amplitudes over 24 hours rose from a baseline value of 96.6mu/l/24hrs (range 50.9-133.9) to 228.9mu/l/24hrs (range 92.4-470.3) on Day 1. This rise was highly statistically significant and did not change further with time. The increment of growth hormone secretion was accompanied by a significant increase in growth velocity from 4.4cm/year (range 3.6-5.1) to 7.6cm/year (range 4.8-11.7) (p=0.02).